

A New Protozoan Ciliate *Entodinium babinum* (Sp.Nov) from the Rumen of Indian Cattle. (*Bos Indicus*)

Abstract

Rumen fluid samples from adult Indian cattle (*Bos indicus*) were collected and observed to study the morphology of rumen ciliate protozoa belonging to genus *Entodinium*. The present paper deals with the morphology of a new species *Entodinium babinum* from the rumen of Indian cattle. The body measurements, frequency distribution and variations of the characters are recorded, critical comments are made on its specific identity, *Entodinium babinum* is characterized by having small size wedge shaped body with a dorsal surface abruptly curved posteriorly and straight ventral surface. Presence of two small caudal spines; thick and wedge shaped macronucleus placed high up near a dorsal lip. of all the known species of *Entodinium babinum* is the smallest species.

Key Words : Cattle, rumen, protozoa, ciliates, *Entodinium*.

Introduction

Protozoa are unicellular animals. The great majority of ciliates are free living, but a number are parasitic. The protozoa placed in the phylum ciliophora possess cilia, cirri or other compound ciliary structures which serve as organelles of locomotion. Two kinds of nuclei are present in all without exception. Nutrition is holozoic or saprozoic. Asexual reproduction is by binary fission or budding and sexual reproduction is by conjugation or autogamy in which micronuclei play an important role. The ciliates of rumen belongs to the families Buetchliidae, Isotrichidae, Paraisotrichidae, Blepharocarythidae, Ophryoscolecidae, and Cyclopostidae. The ciliates vary in size from a few micron to 2 mm or more in length. The anterior and posterior extremities are permanently differentiated, the rumen ciliates are obligate anaerobes.

Ruminants have a very complex ecosystem harboring a variety of microorganisms which are capable of bringing out diverse types of fermentation. Rumen, the largest of the four compartments of stomach in ruminants, serves as a closed fermentation vat in which ingested feed is attacked by the microflora.

The rumen microflora consist of mainly of bacteria, protozoa and fungi, which have a significant role to play in rumen fermentation. Of the total microbial biomass existing in rumen 40 to 80 percent is of protozoal origin (Jouney -1991, Punia et al, 1992), Protozoa living in the rumen are essentially ciliates, flagellates are often less numerous, not well known and are often confused with the flagellate stage of fungi (Jonney - 1988) Fermentation of starch and soluble sugars is regulated by rumen protozoa (Mackie et al 1978) and they are held in controlling acidosis in the rumen. Rumen protozoa are generally proteolytic (Balaraman, 1996).

The ciliates are established in the rumen within three weeks after the birth of a calf (Kurar, 1996) provided that the pH is above 6.0. *Entodinium* population is abundant in the rumen. It increases when the diet is rich in starch. Protozoa contributes about 40 to 60 percent of total hydrolytic enzyme activity in rumen. In ruminants, protozoa were first observed by Gruby and Dalafond in 1843 (Hungate, 1978) Since then a number of protozoal species have been reported in rumen. Subsequently the taxonomic studies on the rumen protozoa was done by various workers in different parts of the world; only a few studies have been carried in domesticated Indian ruminants. Kofoid and MacLennan (1930,1932,1933) in *Bos indicus*, Das Gupta (1935) in Indian Goat, Ajit Banerjee (1955) in Indian Buffalo. There is much scope to do work on the taxonomy of rumen ciliates. The taxonomical work on rumen ciliates of Cattle in India is very scanty. The present research work deals with study of taxonomy of rumen protozoa from Indian cattle.



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Material and Methods

Rumen fluid samples were collected for the present study from Indian adult cattle (*Bos indicus*) slaughtered at abattoirs in Hingoli district of Maharashtra state in India. On the removal of stomach, rumen was slightly punctured and 10ml. rumen fluid was collected in a vial. It was centrifuged and preserved adding 1:1 glycerine:alcohol solution. A drop of this material was taken on a glass slide for observing ciliates in living condition under research microscope. The permanent slides of the samples were made in duplicate, stained by tungstophosphoric haemotoxylein stain. The staining procedure of Krier and Becker, 1987 was followed. The stained slides of ciliates were observed under research microscope for their identification and morphology.

The general features used to classify the rumen protozoa into genus *Entodinium* are as follows : (Dehority - 1993)

1. The Presence of single adoral zone.
2. Lack of skeletal plates.
3. Position of the macronucleus which lies between micronucleus and closest body side.

Body measurements such as length, width, L/W ratio, diameter, length of the nucleus etc. were recorded with an ocular micrometer. Frequency distribution, body shape, location of contractile vacuole, rectum mouth are also recorded.

Taxonomical position of *Entodinium* Stein, 1858.

Subkingdom	:-	Protozoa
Phylum	:-	Ciliphora
Class	:-	Kinetofragminophorea
subclass	:-	Vestibulifera
order	:-	Entodinimorphida
Family	:-	Ophryoscolecidae
Subfamily	:-	Entodniinae
Genus	:-	<i>Entodinium</i>

The following parameters were considered for observation.

- 1) Shape of the body
- 2) Length of the body
- 3) Width of the body
- 4) L/W ratio
- 5) Shape of nucleus
- 6) Length of nucleus
- 7) Position of micronucleus
- 8) Position of contractile vacuole
- 9) Location rectum and anus

Results and Discussion***Entodinium babinum* (sp.nov)
(fig. 1a, 1b)**

During the present study *Entodinium babinum* is recorded as a new protozoan ciliate species belonging to genus *Entodinium* from the rumen of India cattle. Its morphology is described, the body dimensions and other measurements are recorded (Table-1). The variations in the body characters are recorded and critical comments are made on its specific identity. The observations are based on a study of 50 specimens taken at random from different rumen fluid samples.

Morphology

The body of *Entodinium babinum* (sp.nov) is small and wedge shaped. Of all the known species of *Entodinium* it is one of the smallest species. The average body length is 22.38 μm and the maximum

body length is 25.68 μm . Anterior end of body is broad with ventrally inclined, medium size mouth (6.12 μm) The adoral lips are prominent. The L/W ratio is 1.83.

The dorsal body surface is strongly convex in the anterior half. The greatest diameter is in the middle of the body (12.24 μm); from the middle half the dorsal body surface curves more abruptly to the posterior side and is terminated into a very small, triangular dorsal spine (1 μm). The ventral body surface is almost flat and it also terminates into a sharp, pointed small ventral spine (1.65 μm) which is straight and pointed dorsally.

The macronucleus is thick, wedge shaped body, 11.26 μm in length. It is located along the anterodorsal part of the body, extending close from the base of adoral lips passes upto middle and the body. The anterior tip of macronucleus is broad and rounded (2.52 μm), while the posterior end is narrow and pointed (1.50 μm). The micronucleus is small spherical body located on the left ventral edge of the anterior third of macronucleus. Contractile vacuole is located anterior to the left of macronucleus.

The endoplasmic sack is surrounded by a prominent boundary layer at some distance from the ventral surface posteriorly. Thick ectoplasm is present in this area of ventral side posteriorly, while slightly thick ectoplasm is present near the spines. Rectum is transverse slit located between the two spines. Its position is to the ventral side posteriorly.

Comments

Entodinium babinum (sp.nov) is the smallest species of *Entodinium*. This species comes close to *E.laterospinum* Kofoid and MacLennan, 1930, *E.brevispinum*, Kofoid, MacLennan 1930, *E.nanellum* Dogiel, 1922, in its body size.

This species differs from *E.laterospinum* in having two caudal spines, one straight, ventral spine and the other small, triangular dorsal spine; whereas *E.laterospinum* has a single ventral spine, which is long, curved, deflected at an angle of 30° to the right from the main axis of the body.

The present species also differs from *E.brevispinum* by having two caudal spines; the ventral spine is straight as against a single curved ventral spine of *E.brevispinum*. The ventral body surface of this species is straight as against ventral surface having greater curvature posteriorly giving a humpback appearance in *E.brerispinum*. The micronucleus of this species is small spherical body as against ellipsoidal micronucleus of *E.brevispinum*.

The species differs from *E.nanellum* in having two caudal spines as against smooth and rounded posterior end without spine in *E.nanellum*. It also shows the difference in body shape, in having a small, slender and wedge shaped body as against small, ovoidal body of *E. nanellum*. The position of macronucleus is high up in the present species than *E.nanellum*.

The comparative table indicating the body dimensions of closely related species with are described herein with that of *E.babinum* (sp.nov) are indicated in (Table-2)

References

1. Banerjee, Ajit Kumar (1955), Studies on parasitic ciliates from Indian ruminants. *Proc.Zool. Soc.*, 8(2) : 87-100.
2. Das Gupta, Matiranjan (1935). Preliminary observations on the protozoan fauna of the rumen of the Indian goat. *Copra hircus Linn. Arch. Protistenk*, 85(2) : 153-172.
3. Dehority, B.A. (1974). Rumen ciliate fauna of Alaskan moose. musk-ox and Dall mountain sheep. *J.Protozo.*, 33(3) : 416-421.
4. Dehority, B.A. (1978). Specificity of rumen ciliate protozoa in cattle and sheep. *J.Protozool.*, 25(4) : 509-513.
5. Dehority, B.A. (1986). Rumen ciliate fauna of some Brazilian cattle : occurrence of several ciliates new to the rumen including the cytoposthid *Parentodinium africanum*. *J. Protozool.*, 33(3) : 416-421.
6. Dehority, B.A. (1993). Laboratory manual for classification and morphology of rumen ciliate protozoa. CRC Press Inc., pp. 1-120.
7. Dogiel, V. A. (1922). Die Artbildung in der Infusorien families ophryoscolecidae. *Arch. Russe. Protistol.* 2:89-104.
8. Gruby and Dalafond (1843). sur des animalcules se developpant dans lestomac et dans les intestins pedant la digestion des animaux herbivores et carnivores. *Conpt. Rend. Acad. Paris.* 17: 1304-1308
9. Gocmen, B. and Oktem, N. (1996). New rumen ciliates from Turkish domestic cattle (*Bos taurus L.*) presence of *Entodinium dalli* Dehority, 1974 with a new forma *E.dalli*, f. *rudidorospinatum n.f.* and comparisons with *Entodinium williamsi n.sp.* *Europ. J.Protistol.*, 32:513-622.
10. Gocmen, B and Oktem, N. (1999). Taxonomical status of the rumen ciliate, *Entodinium longinucleatum* Dogiel, 1925 in domesticated cattle. *Tr. J. Zoology*, 23 (supp.2) : 465-471.
11. Hungate, R. E. (1978), The Rumen protozoa, in Krier, P.P. ed. *Parasitic protozoa*. Academic press – Jnc, New York 2:655-695.
12. Imai, S. (1986). Rumen ciliate protozoal fauna of Zebu cattle (*Bos taurus indicus*) in Shrilanka with the description of a new sp. *Diplodinium sinhalicum*. *Zool. Sci. (Tokyo)*, 3(4) : 699-706.
13. Imai, S. (1988), Ciliate Protozoa in the rumen of Kenyan zebu cattle *Bos taurus indicus* with the description of four new sp. *J. Protozoa* 35 (1) : 130-136.
14. Imai S. and ogimoto, K. (1984), Rumen ciliate protozoal fanna and bacterial flora of the Zebu cattle (*Bos indicus*) and the water buffalo (*Bubalus bubalis*) in Thialand, Jpn, *J. Zootech. Science* 55:576-583.
15. Ito, A, Imai S (1990), Ciliate Protozoa in the rumen of Holstein Friesian cattle in Japan *Zoology Science* 7 (3) : 449-458.
16. Jouany, J. P. Demeyer, D.J. and Grain J. (1988) Effect of defaunating the rumen. *Animal Feed Science and Technology* 21:229-265.
17. Kulkarni, S.A. and Kshirsagar, H.S. (2004). Two new species of protozoan cilites from the rumen of Indian Cattle (*Bos indicus*) *Asian Jr. of microbial. En. Sc.* 6 (1) 2004. 123-125.
18. Kulkarni, S.A. and Kshirsagar, H.S. (2005). Description of two new protozoan species, *Entodinium biconvexum*. (sp.nov.) and *Entodinium flgi* (sp. nov.) from the rumen of Indian cattle (*Bos indicus*) *Asian Jr. of microbial. En. Sc.* 7 (3) 2005. 491-494.
19. Kulkarni, S.A. and Kshirsagar, H.S. (2006). Taxonomical study of the rumen protozoan ciliate *Entodinium ciculum* (Dehority, 1979) from the rumen of Indian Cattle (*Bos indicus*) *Asian Jr. of microbial. En. Sc.* 8 (1) 2006. 41-43.
20. Kulkarni, S.A. and Kshirsagar, H.S. (2008). Description of a new protozoan ciliate *Entodinium wedgunum* (sp.nov.) from the rumen of Indian Cattle (*Bos indicus*) *Natl. J. Life Sci.* 5 (3) 2008 (115-119).
21. Kulkarni, S.A. (2012). Description of a new protozoan ciliate *Entodinium triangulospinum* (sp.nov.) from the rumen of Indian cattle (*Bos indicus*) *AARJMD*, 1 (3) 2012 : 12-17.
22. Kulkarni, S. A. (2013), Description of *E.rectangulatum f Caudatum*, (Lubnisky, 1957) from the stomach of Indian cattle. *Periodic research*, 1 (III) : 2013: 32-36.
23. Kofoid, C.A.; and MacLennan, R.F. (1930), Ciliates from *Bos indicus* L.1. The genus *Entodinium stein*. *Univ. Calif. Publ. Zool.* 33(22) : 471-544.
24. Lubinsky, G. (1957), Studies on the evolution of the Ophryoscolecidae I. A new species of *Entodinium* with "caudatum"lobospinosum and dubardi forms and some evolutionary trends in the genus *Entodinium*. *Can. J.Zool.* 35 : 111-133.
25. Misra, S. K. ; P.K. Das, and G. P. Mohanty (1972). The protozoan fauna of the rumen and reticulum of Indian cattle, *Indian Vet. J.* 49 : 463 – 469.
26. Sanghai, P.K., Kshirsagar, H.S. and Kulkarni S.A. (2010) Description of two new protozoan species *Diplodinium spericulatum* sp.nov. and *Diplodinium posterotriangulatum* sp.nov. from the rumen of Indian cattle (*Bos indicus*) *Asian Jr. of microbial. En. Sc.* 12 (1) 2010. 205-208.
27. Selim, H. M., Imai, S., Sheik, A.K. Attiq, H., Okamoto E. Miyagawa, E. maede, Y. (1999), Rumen ciliate protozoal fauna of Native Sheep, Freisian cattle and dromedary camel in Libya. *J.vet med Science.* 61(3) : 303-305.
28. Tung, K.C. wang, J.S., Shyu, C.L. (1989) Detection of rumen ciliates of cattle in Taiwan. *J.Agriculture and forestry.* 38 (1) 147-162.
29. Wertheim, P (1935), A new ciliate *Entodinium bovis n.sp.* from the stomach of the *Bos taurus L.* with the revision of *E. exiguum*, *E.nanellum*, *E.simplex*, *E.dubardi* and *E.Parurom*. *Parasitology* 27:226-230.

Table – 1
The Body Dimensions and other measurements of *Entodinium babinum* (sp.nov) are given below.

(All the measurements in microns)

Sr. No.	Parameters	Minimum	Maximum	Average
1.	Body			
	Length	17.12	25.68	22.38
	Width	10.70	14.98	12.24
	Length width ratio	1.60	1.71	1.83
2.	Macronucleus			
	Length	6.42	17.12	11.26
	Percent length of body	37.50	66.67	50.31
	Dia. Ant. end.	2.14	4.28	2.52
	Dia. post. end.	1.28	2.14	1.56
3.	Mouth	4.28	8.56	6.12
4.	Spine	0.86	3.00	1.65

TABLE – 2
Comparative Body Dimensions of *Entodinium babinum* (sp.nov) with closely related species of Entodinium given by earlier workers and the present dimensions (in microns)

Param-eters	Authors			
	<i>E.brevispinum</i> Kofoid and MacLennan (1930)	<i>E.nanellum-Dogiel</i> (1922)	<i>E.laterospinum</i> Kofoid & MacLennan (1930)	<i>E.babinum</i> (sp.nov) (2013)
Length	24-30 (28)	22-32 (28)	25-32 (28)	17.12-25.68 (22.38)
Width	15-18 (16)	12-18 (16)	14-19 (16)	10.70-14.98 (12.24)
L/W ratio	1.5-2.00 (1.70)	1.5-2.0 (1.76)	1.56-1.86 (1.71)	1.60-1.71 (1.83)
Macronucleus	12-18 (13)	11-21 (15)	12-20 (15)	6.42-17.12 (11.26)
Spine	1-2 (1)	--	4-9 (7)	0.85-3.00 (1.65)

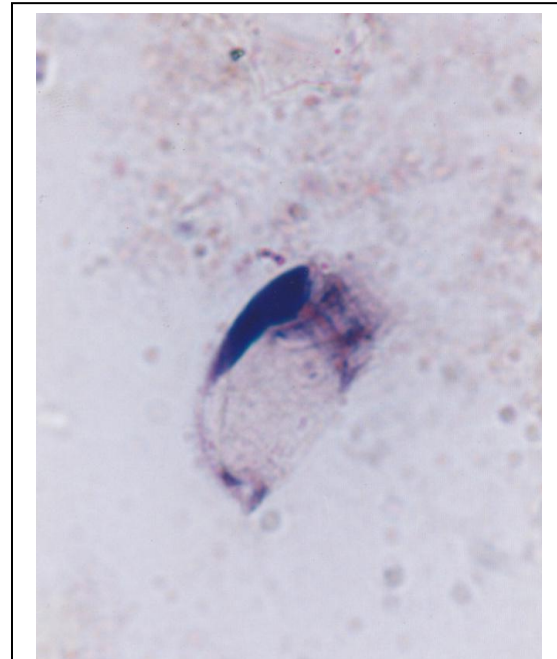


Fig. 1a)
Photograph of Entodinium babinum (sp.nov)

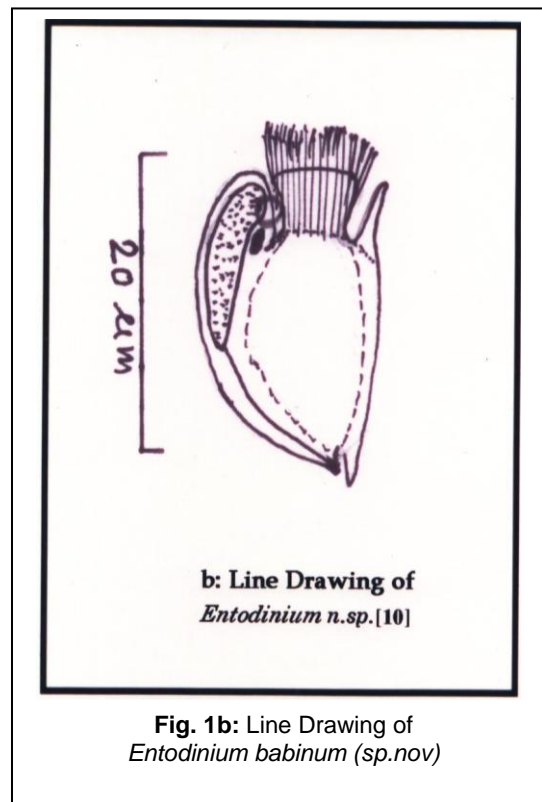


Fig. 1b: Line Drawing of *Entodinium babinum* (sp.nov)